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Medical Progress

Diseases of Phenylalanine Metabolism

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Continuing investigation of the system that hydroxylates phenylalanine to tyrosine has led to new insights into diseases associated with the malfunction of this system. Good evidence has confirmed that phenylketonuria (PKU) is not caused by a simple lack of phenylalanine hydroxylase. Dihydropteridine reductase deficiency as well as defects in biopterin metabolism may also cause the clinical features of phenylketonuria. Furthermore, these diseases do not respond to the standard treatment for phenylketonuria.

IN 1934 the Norwegian physician and biochemist, Asbjorn Fölling described a disorder of phenylalanine metabolism which has since been called phenylketonuria or PKU. His observations led him to suspect that mentally defective persons with this disease differed biochemically from others. The reasoning, in turn, initiated an entirely new approach toward investigating the causes of mental retardation. PKU was the first disorder of amino acid metabolism for which a specific enzyme defect was established. Good evidence now exists that inborn enzymatic defects are the underlying causes of nearly 150 metabolic disorders and others very likely will be discovered. Previously considered a single enzymatic illness, the diseases of phenylalanine metabolism are now accurately recognized as a complex spectrum of disorders.

The Hydroxylating System

Fölling showed that persons with PKU have elevated levels of phenylalanine in the blood and that they excrete phenylpyruvate in their urine.[1,2] In 1947 Jervis conducted a series of experiments that gave a clearer understanding of the metabolic block and correctly identified the affected reaction in phenylketonuria.[3] Jervis administered phenylalanine to normal animals, as well as to humans, and noted a prompt increase in serum levels of tyrosine; when he gave phenylalanine to phenylketonuric patients, there was no increase in tyrosine. Therefore, he postulated that the block was in the system responsible for hydroxylating phenylalanine to tyrosine (Figure 1).[4,5] He later presented direct evidence supporting his hypothesis by showing that in a liver specimen from a phenylketonuric patient, phenylalanine was not converted to tyrosine.[6] If the system that catalyzes the hydroxylation of phenylalanine had been composed of only phenylalanine hydroxylase, Jervis's work would have adequately explained this aspect of PKU. However, in the last few years, researchers have shown that Jervis's hypothesis was grossly oversimplified. Actually, the phenylalanine hydroxylating system is complex, consisting of several essential components. The lack of any one of these can cause a malfunction of the entire system. The earliest indication of the complexity of this system was provided by Mitoma in 1956[7] and Kaufman in 1957.[8] Some years later Kaufman showed that the coenzyme biopterin also played an essential role in the reaction.[9]

Figure 2 shows the scheme for the complex hydroxylating system. Phenylalanine and tetra-

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hydrobiopterin are oxidized to tyrosine and dihydrobiopterin, respectively. Tyrosine and TPNH (nicotinamide adenine dinucleotide phosphate, reduced) mediate the reduction of the dihydrobiopterin, thereby regenerating tetrahydrobiopterin. The role of the reductase is to keep the pterin cofactor in the active tetrahydro form.

The dihydrobiopterin produced from the tetrahydrobiopterin during the hydroxylation reaction is an extremely unstable quinoid derivative.[10] Unless it is rapidly reduced back to the tetrahydro level, it rearranges by tautomerization to a more stable isomer, the 7,8 dihydrobiopterin,[10,11] which cannot be reduced by dihydropteridine reductase.[12] This compound can be brought back into the system by still another enzyme, dihydrofolate reductase.[13] This enzyme probably serves to salvage the 7,8 dihydrobiopterin that may be formed from the quinoid compound in any circumstances where the dihydropterdine reductase-catalyzed reaction is not fast enough to keep pace with the hydroxylase.[14] In addition, it has been shown that certain phospholipids, such as lysolecithin, when in the presence of tetrahydrobiopterin, can stimulate purified hydroxylase 30-fold to 50-fold.[15] A second reaction-stimulating product called phenylalanine hydroxylation-stimulating protein has been reported.[14,16,17] This protein also requires tetrahydrobiopterin as a cofactor.

To summarize, four factors are directly involved with the phenylalanine hydroxylation reaction: phenylalanine hydroxylase, dihydropteridine reductase, tetrahydrobiopterin and dihydrobiopterin. Finally, and perhaps playing a less dramatic role, is a fifth factor: dihydrofolate reductase. In addition, phospholipids and phenylalanine hydroxylase-stimulating protein appear to initiate the hydroxylating reaction in the presence of tetrahydrobiopterin.

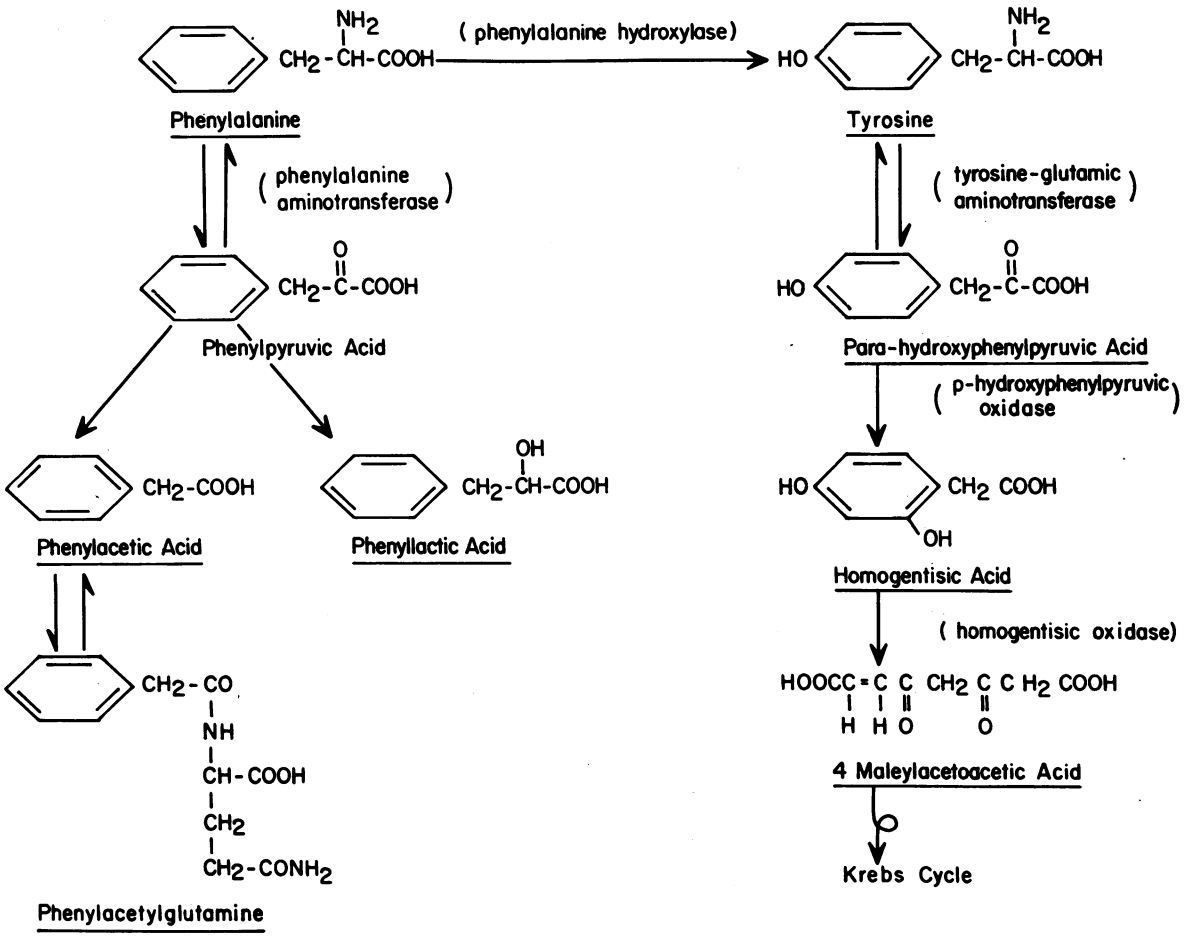

Figure 1.—A general scheme of some of the pathways of phenylalanine and tyrosine metabolism. (Adapted from Lehninger AI[4] and Koch R et al.[5])

Dihydropteridine Reductase Deficiency

There have been several reports of phenylketonuria in which neurologic deterioration has occurred despite adequate dietary restrictions to control levels of phenylalanine in the blood.[18-25] Using loading studies to determine the degree of hyperphenylalaninemia has limitations because they indicate the degree of dysfunction of the hydroxylating system as a whole, but give no indication of the function of individual components.

A child with dihydropteridine reductase deficiency will present exactly like a child with phenylalanine hydroxylase deficiency, but his or her clinical situation will continue to grow worse rather than better with dietary control of blood phenylalanine.[22] The reductase can be assayed from cultured skin fibroblasts and the procedure may be advisable in the initial diagnosis of phenylketonuria. It should be indicated in any child who is doing poorly or in whom seizures develop while on a phenylalanine-restricted diet.[24,25]

Defects in Biopterin Metabolism

Reports of defects in the metabolism of biopterin have begun to appear in the literature.[19,23] Children with this disorder have symptoms similar to those of dihydropteridine reductase deficiency, and progressive neurologic deterioration occurs in spite of adequate dietary control of phenylalanine in the blood. Biopterin derivatives from serum and urine can be measured[26] and should be considered in diagnosing unusual cases.

The same biopterin cofactor system used in the phenylalanine hydroxylation reaction is also used by neural tissue in the synthesis of the amino transmitters dopamine, norepinephrine and epinephrine, as well as in the synthesis of serotonin from tryptophan.[19,20] Therefore, a deficiency of biopterin or the enzyme dihydropteridine reductase would interfere with conversion of phenylalanine to tyrosine as well as impair production of neurotransmitters in the brain. Such a defect would be expected to cause severe neurologic disease. Phenylalanine restriction would correct the hyperphenylalaninemia without affecting the block in transmitter production, and it is unlikely to benefit the neurologic symptoms.

The Hydroxylating System in Newborn Infants

Studies on the capacity of the phenylalanine hydroxylating system of neonates have produced conflicting results. Some investigators have found that after administration of phenylalanine there is increased excretion of tyrosine in both full-term and premature neonates,[27] and that in premature infants tests may show normal phenylalanine tolerance;[28] these are consistent with an active hydroxylating system. There have been other reports of elevated levels of blood phenylalanine in premature and full-term neonates and decreased tolerance for phenylalanine in normal neonates.[29-31]

There is ample evidence that phenylalanine

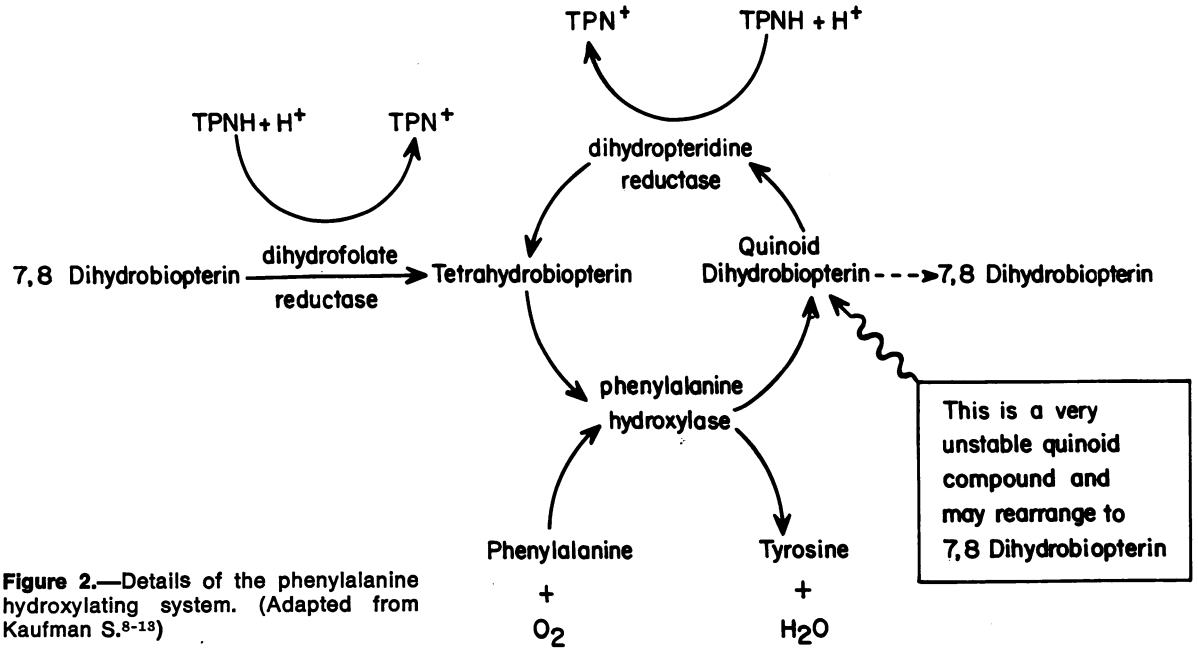

Figure 2.—Details of the phenylalanine hydroxylating system. (Adapted from Kaufman S.[8-13])

hydroxylase activity in the human liver begins at about the 8th week[32] after conception and approaches an adult level of activity at about the 13th[33,34] week; however, the activity appears to decrease in the third trimester.[35]

The Isoenzymes of Phenylalanine Hydroxylase

In the early 1960's it was discovered that the same enzyme could exist in several different forms within a single tissue. This discovery led to new information concerning the nature of enzymes, as well as enzyme changes in disease.[36] Like many other enzymes, phenylalanine hydroxylase is now known to occur in several molecular forms.

The possibility of multiple forms of phenylalanine hydroxylase was suggested in the late 1960's to explain the various types of hyperphenylalaninemia.[37-39] Bessman and Huzino were able to separate phenylalanine hydroxylase into two isoenzymes.[40] Kaufman and Fisher also reported two forms of the isoenzyme in both human and rat livers.[41-43] In 1972 Barranger and colleagues[44] described three forms of phenylalanine hydroxylase in rat liver and in 1976, Barranger characterized the three isoenzymes of phenylalanine hydroxylase (pi, kappa and upsilon) in humans as well.[45] Parker and co-workers[46] reported the results of examinations in 12 healthy adults and 10 children with classic phenylketonuria; 8 of the 12 adults had three isoenzymes; 3 had an absence of the kappa fraction, and one person was missing both pi and upsilon fractions. None of the children with classic phenylketonuria had more than 5 percent of the activity of the normal adults and no more than one isoenzyme was ever found in any of these children.[47]

Isoenzymes are the result of variation in one

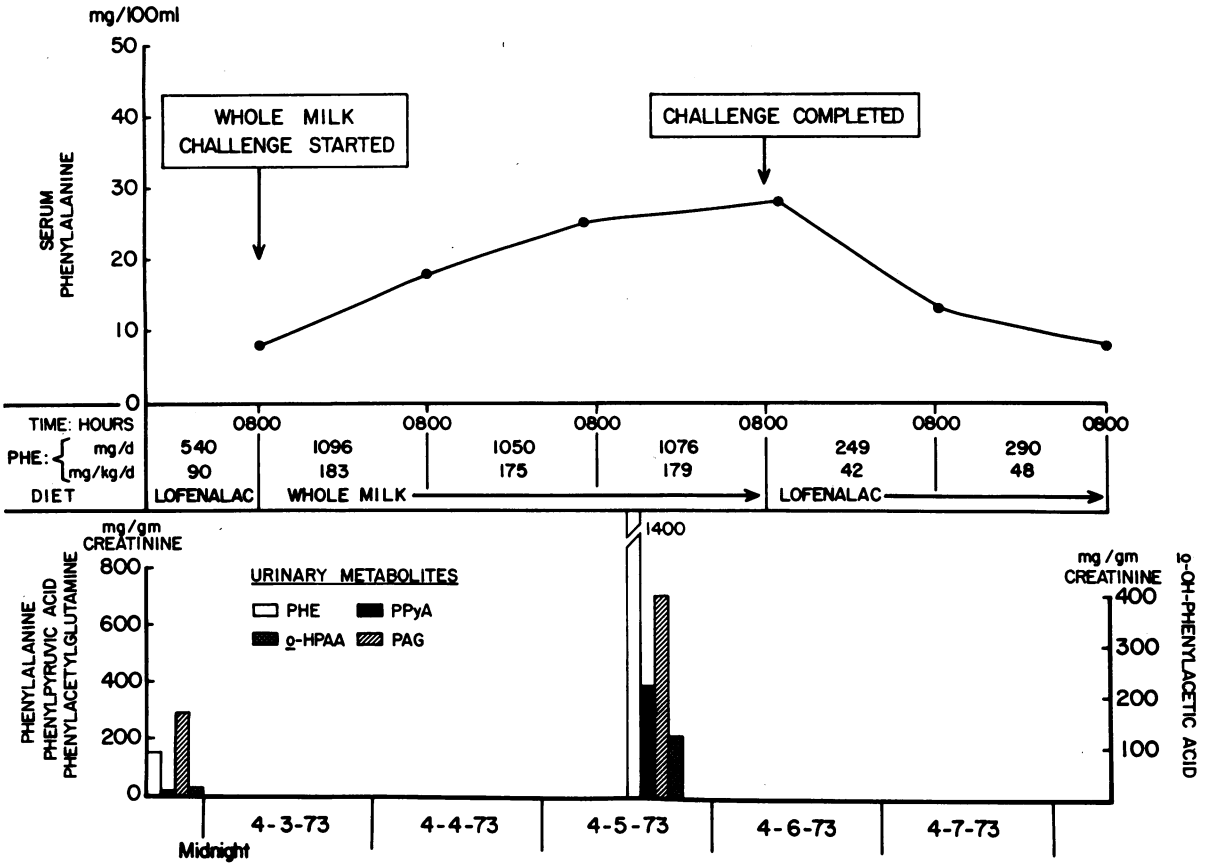

Figure 3.—A typical phenylalanine tolerance test using an oral intake of whole milk calculated to give 180 mg per kg of body weight per day of phenylalanine. Initial baseline studies of blood for serum phenylalanine and urine for phenylalanine metabolites are obtained. After challenge has begun, blood and urine specimens should be obtained every 12 hours for biochemical studies. The challenge is terminated after 72 hours. (It should be noted that in this study the serum phenylalanine level rose well above 20 mg per dl and remained there longer than 24 hours; it was accompanied by an increase of all the urinary metabolites.)

of two basic themes in protein structure, either subunit composition or amino acid sequences. Isoenzymes resulting from differences in amino acid sequences presuppose a separate and distinct mechanism for the synthesis of each form. It should be noted that such a mechanism need not be genetic or pretranscriptional in origin, but can also represent a posttranslational modification. In any event, all data at this time suggest that the isoenzymes of phenylalanine hydroxylase are three clearly different forms.[45]

Isoenzymes probably play a much more important role in explaining the wide clinical variation of the hyperphenylalaninemias[31,35,48-50] than do the extremely rare disorders, pteridine reductase and biopterin.

Definition of Phenylketonuria

The normal blood level of phenylalanine in humans is 1 to 3.5 mg per dl. Any level above 3.5 mg per dl can be called hyperphenylalaninemia. At one end of the spectrum is phenylketonuria (Fölling disease, PKU and classic PKU are synonymous terms), with its attendant mental and motor retardation. At the other end of the spectrum are the mild hyperphenylalaninemias which produce few if any detectable ill effects but which, nonetheless, should receive attention because there is evidence that few adults with elevated phenylalanine have completely normal intelligence.[51,52]

The literature is in agreement that classic phenylketonuria should be treated although, as yet, no clear definition exists.[5,53-59] Some authors refer vaguely to treating children in whom serum phenylalanine levels are greater than 25 mg per dl.[60] Others state that any child with blood phenylalanine levels greater than 15 mg per dl, especially when they are accompanied by urinary metabolites of phenylalanine, should be treated.

For purposes of consistency, we have chosen the definition used by the Collaborative Study of Children Treated for Phenylketonuria.[62] They calculate a loading dose of L-phenylalanine from a *normal* diet which is about 180 mg per kg of body weight per day. Figure 3 illustrates a typical loading study, which can be defined as follows: In two determinations, the serum phenylalanine level is 20 mg per dl or above in two blood specimens drawn at least 24 hours apart. From the same blood specimens, two determinations of serum tyrosine level show values below 5 mg per dl, accompanied by elevated levels of the metabolites of phenylalanine in the urine. In normal subjects given a single dose of L-phenylalanine, levels peak rapidly and return to the baseline within four hours. Likewise, urinary excretion of phenylalanine and its metabolites (Figure 3), phenylpyruvic acid, orthohydroxyphenyl acetic acid and phenylacetyl glutamine, increase very little in normal children.[55]

In the absence of a referral center, and if the sophisticated loading studies just described are not available, then any child with a persistent serum phenylalanine level greater than 10 mg per dl should be treated.

The term *variant* is found throughout the literature and is frequently applied to those forms of hyperphenylalaninemia that physicians do not consider to be cases of classic PKU. Unfortunately, the use of this term is often interpreted to mean that treatment is unnecessary. With the elucidation of the components of the hydroxylating system, the term variant has begun to be applied to those forms of hyperphenylalaninemia and presumably could be applied to the isoenzyme deficiencies as well. It is hoped that the term will eventually be replaced by a more specific diagnosis.

Screening

It is necessary to test newborn infants if a diagnosis is to be made before signs and symptoms appear, and in time for a low-phenylalanine diet to be most effective.[63] Screening methods originally were based on testing for the presence of phenylpyruvic acid in urine. This proved adequate for older children and adults, but inadequate for very young infants who may not begin to excrete phenylpyruvic acid for several weeks.[64,65] In addition, because phenylpyruvic acid is relatively unstable and oxidizes easily a diagnosis may be missed if the test is not done promptly.

There are many methods for determining the presence of phenylalanine in the blood.[66] A fluorometric method based on the formation of a fluorescent substance when phenylalanine is heated with ninhydrin in the presence of leucylalanine is now widely used.[67]

Paper chromatography, thin layer chromatography and gas chromatography have the advantage of showing small increases in phenylalanine as well as other amino acids; however, these methods are time consuming and appear to be impractical for mass screening programs.

The most popular method used for mass screen-

ing has been developed by Guthrie and Susi.[68] Discs are punched from filter paper that has been saturated with blood and then allowed to dry. These uniform discs are incubated on a tray of agar seeded with Bacillus subtilis and containing 3-2-thienylalanine, an inhibitor of the organism's growth. As the phenylalanine from the blood spreads from each disc onto the medium it reverses the inhibition of bacterial growth, thereby yielding an area of bacteria that can be measured and compared with that obtained from discs containing known amounts of phenylalanine. This simple, effective method is now used to screen newborn infants throughout the United States and in many parts of the world.[69]

The possibility of missing a diagnosis is greatest in neonates screened very shortly after birth.[54,70] In a study of infants in whom a diagnosis of phenylketonuria was later established, the phenylalanine concentration during the first three days of life was substantially lower than in those screened later.[70-72] It appears desirable to delay newborn screening as long as is safely possible[73] and, for those infants in whom a negative result is obtained before 3 days of age, a second test should be seriously considered at the first well-baby visit. All initial serum phenylalanine levels greater than 4 mg per dl should be further investigated.

Treatment

Reducing phenylalanine intake by dietary management has been the mainstay of therapy since Bickel and co-workers first described it in 1954.[74,75] Independently and almost simultaneously, Armstrong's group in the United States[76] and Woolf and his colleagues in London[77] also began using a low-phenylalanine diet. The goal of the diet is to reduce blood phenylalanine to acceptable clinical levels that are as near to normal as possible. Essentially, the dietary regimen includes a well-balanced amino acid mixture with either a very small amount or no phenylalanine at all. The amino acid mixture provides most of the necessary protein because the patient is not ordinarily allowed to eat high-protein foods such as meat, eggs and milk products. However, natural foods that are low in phenylalanine such as vegetables may supplement the diet. The amount of these foods allowed depends largely on each person's phenylalanine requirement.[61,78,79] The minimum level of blood phenylalanine below which treatment is unnecessary remains in question. Most physicians agree that treatment is necessary for levels above 20 mg per dl, and many clinics treat infants if their levels of phenylalanine are persistently greater than 15 mg per dl and if abnormal metabolites are present in the urine.[79,80] Children with concentrations below 10 mg per dl are usually not treated, although there is concern that there may be slight retardation associated with even these small elevations of phenylalanine, especially when they occur during the first few months of life.[81]

There are several preparations on the world market manufactured for use in managing patients requiring a low-phenylalanine diet. In the United States those most widely used are Lofenalac and the newer Product 3229,[82] both manufactured by the Mead Johnson Company. Albumaid X P,[83] is available from Ross Laboratories as PKU-aid; Aminogran, Cymogran and Minafen are amino acid mixtures made in Europe, and Lophe Milk and Phenytola are made in Japan.

Dietary treatment is not effective in those rare patients whose biochemical features result from causes other than phenylalanine hydroxylase deficiency.[21,23,24,84-87] In some cases good response to L-dopa, L-5-hydroxytryptophan and carbidopa have been reported.[23,87,88] The administration of pterin has also been suggested: however,[85,89] there is considerable concern about the efficacy of this treatment.[90]

The Collaborative Study

In 1967 a national prospective collaborative study of children treated for phenylketonuria was initiated. Its goal was to determine the effect of dietary treatment on the mental, physical and psychosocial development of children.[62,91] It was designed to overcome many of the problems of previous studies such as lack of documentation of diagnosis, laboratory variability from one clinic to another, small sample size, lack of random design, bias selection of persons placed in institutions, and bias based on the intelligence and social class of the parents.[92]

Children who were identified as having phenylketonuria were randomly assigned to one of two treatment groups. An attempt was made to maintain blood phenylalanine levels between 1.0 and 5.4 mg per dl in the first treatment group and between 5.5 and 9.9 mg per dl in the second group. The investigators have found it extremely difficult to maintain levels in the children within these arbitrary limits because these levels have

tended to drift upward. The latest report indicates that 203 children are in the study.[63]

This same publication reported the intelligence quotients of 111 of the children in the study who had reached the age of 4 years.[63] The mean Stanford Binet intelligence quotient was 93, which is slightly below the mean for the normal population, although well within one standard deviation. Furthermore, those children for whom dietary treatment was initiated during the first month of life scored a mean intelligence quotient of 95, compared with 85 for those who were initially treated at 31 to 65 days after birth. Overall, the results are encouraging, and lessen fears that the diet is harmful or ineffective.

Discontinuation of Diet

The relationship between reducing phenylalanine in the blood and minimizing mental retardation in children with phenylketonuria has been well established;[5,56,93-100] however, determining the most efficacious time to discontinue the diet remains a serious problem. Some clinicians suggest stopping the diet as early as age 4[94] or 5 while others are much more conservative.[5,102] Some investigators feel that mental deterioration occurs even in older children if blood phenylalanine levels are allowed to increase as a result of an unrestricted phenylalanine intake,[100-104] but others disagree.[79,85,105-107]

Maintaining a child who both appears *and* feels normal on a special diet for years is a heavy burden for the child and the family. Aside from financial considerations regarding maintenance of the dietary regimen, important noneconomic factors exist as well. The necessity of eating *special* food and the stress of being an outsider at parties or group activities where other children have no dietary restrictions often contribute to poor social and behavioral adjustment in these children.[98,107,108] The special diet makes meal planning difficult and can cause tension during those times the family is together. Therefore, it is beneficial to take children off the diets when safely possible. If, on the other hand, there is continued risk of mental deterioration,[109] the children should remain on treatment.

The Collaborative Study is considering the effects of discontinuing the low-phenylalanine diet by randomly selecting children for continuation or discontinuation at the age of 6 years. At present the number of children is still too small to detect pronounced differences between the two groups.[110]

Maternal Phenylketonuria

The effects of maternal phenylalanine elevation on offspring were first suggested by Dent in 1956.[111] His hypothesis has since been confirmed by the findings of numerous studies.[112-150] Early diagnosis and treatment of the disorder has produced an increasing population of women with hyperphenylalaninemia who are approaching child-bearing age; therefore, this issue has become important.

Pueschel[78] reviewed the world literature[111-150] on maternal phenylketonuria and found 61 phenylketonuric mothers who gave birth to 197 children. Of these children, 17 were phenylketonuric; however, only 32 of the 197 had normal intelligence. Eight of the 61 mothers with classic phenylketonuria had attempted or carried out some dietary treatment during part of their pregnancies. Three of these mothers gave birth to nonphenylketonuric children with normal intelligence.

At the 14th annual meeting of the Collaborative Study of Children Treated for Phenylketonuria,[110] Mabry presented an updated review of the world literature[151-170] to which we have added several cases known to us or in additional literature[171] (Table 1). The mothers were divided into two groups: those whose blood phenylalanine levels were greater than 20 mg per dl were designated as having PKU and those whose blood phenylalanine levels were less than 20 mg

TABLE 1.—*Summary of the Outcomes of Pregnancies of 87 Women With Hyperphenylalaninemia*

	PKU	*Non-PKU*	*Unexamined*	*Live Births*	*Spontaneous Abortions*	*Number of Pregnancies*	*Unexplained Infant Deaths*	*Normal IQ*	*Seizures*	*Microcephaly*	*Growth Retardation*	*Congenital Anomalies*
65	Mothers with PKU (phenylalanine levels greater than 20 mg per dl)											
	21	150	25	196	41	237	27	17	20	76	25	28
22	Hyperphenylalaninemic mothers (phenylalanine levels less than 20 mg per dl)											
	6	54	6	65	13	79	5	8	2	23	4	7
87	27	204	31	261	54	316	32	25	22	99	29	35

PKU = phenylketonuria

per dl were designated as having hyperphenylalaninemia. There were 261 live births and 10 percent of the infants from each group were phenylketonuric. There were 41 spontaneous abortions and 27 unexplained infant deaths in the PKU group representing a loss of 29 percent. In the hyperphenylalaninemic group there were 5 unexplained infant deaths and 13 spontaneous abortions which represented a loss of 23 percent. Of extreme concern was the high percentage of microcephalic children born to this group of mothers. Only 9 percent of the children in the PKU group and 12 percent of those born to hyperphenylalaninemic mothers had normal intelligence. Of the eight women in the PKU group who were receiving dietary treatment for the last half of their pregnancies,* three had normal children. One of the hyperphenylalaninemic mothers was treated from the 21st to the 39th week of her gestation[140] and she gave birth to a normal child. Although this is encouraging, it does not provide evidence that a diet restricted in phenylalanine is sufficient to produce intellectually normal children.

In addition, there is growing evidence that heterozygous asymptomatic mothers may provide unfavorable circumstances for normal fetal development.[172-175] Bessman[176] has proposed that genetic deficiencies in nonessential amino acid synthesis may be important causes of mental retardation. He postulates that a person who is genetically deficient in the synthesis of any of the 12 nonessential amino acids, nevertheless requires that amino acid in the diet just as a healthy person requires any essential amino acid. Because the late stage of fetal development is the period when the brain grows most rapidly, and because a deficiency in any single amino acid causes diminished protein synthesis, Bessman proposes that mental retardation occurs during this time because a heterozygote mother is unable to deliver an appropriate amount of the nonessential amino acid (tyrosine in this case) to her fetus that, in turn, is unable to correct for the deficiency due to its own genetic constitution. Supplementing the diets of heterozygous phenylketonuric mothers with tyrosine has been suggested as a way of solving this problem.

Identification of Heterozygotes for Phenylketonuria

It would be useful to be able to identify heterozygotes for PKU. Many systems have been proposed using loading studies or serum levels of phenylalanine, or both, under controlled conditions.[177-180] Although complete discrimination between heterozygotes may not be achieved,[181] the methods are sufficiently sensitive to give a high probability of discrimination between homozygotes and heterozygotes, which is useful in counseling. In addition, liver biopsies can be used for direct enzyme assay.

Anomalies

The occurrence of major anomalies in infants born in a normal population has been estimated at 2 percent and the incidence of at least one minor anomaly at 14.7 percent.[182] Johnson and co-workers,[183] reporting on 150 cases of phenylketonuria, found an overall incidence of anomalies of 9.3 percent, a rate which is not substantially different from that found in the general population. The only exception was an increased incidence of pyloric stenosis which has previously been noted.[184,185] The high incidence rate suggests that infants with pyloric stenosis should be considered for a repeat serum phenylalanine test. The high incidence also indicates a need for physicians to consider the possibility of various metabolic diseases such as hyperphenylalaninemia when evaluating neonates with excessive vomiting.

Phenylketonuria has been reported in conjunction with a variety of disorders including the Down syndrome,[186,187] the Klinefelter syndrome,[188] megacolon,[189,190] scleroderma[191,192] and Fahr disease.[24] It has also occurred with other genetic diseases: neurofibromatosis,[193] hypophosphatasia,[194] familial hypercalcemia,[195] cystathioninuria,[196] muscular dystrophy,[197,198] hyperammonemia[199] and Schilder leukodystrophy.[200] Neuronal lipofuscin lipidosis[201] and alpha-hydroxybutyric aciduria[202] have been reported in patients with biochemical features of phenylketonuria. Whether these represent a coincidence in the presence of two disorders or whether, like dihydropteridine reductase deficiency, these conditions produce biochemical features similar to those of phenylketonuria, is unknown.

The Team Approach

Hyperphenylalaninemia states are rare, and a practicing physician may never encounter a form of the disorder in his lifetime. The diagnosis of the many states of hyperphenylalaninemia is difficult to make and dietary management is time consuming. Therefore, a team approach in treatment

*References 121, 130, 139, 148, 149, 158, 163, 165

is highly recommended.[5,107,206] Ideally, a psychologist should be available for testing,[203] as well as a counselor to assist the family.[204] A qualified dietitian familiar with the phenylalanine content of various foods is essential.[61,79,205] University centers are often better equipped to treat hyperphenylalaninemia. Four or five children may be examined in a single day and sophisticated laboratory procedures are available for diagnosis and follow-up. To be sure, phenylketonuric children also contract the same illnesses as other children and require the same type of primary care as do the others. Thus, for optimal medical care, the specialty clinic should maintain good communication with primary care physicians to assure the best possible continuity of treatment.

Pathophysiology

Phenylalanine ingested in food is distributed in three ways: it is used directly for body protein synthesis, is metabolized or it remains in the free amino acid pool in the blood, plasma and tissues.[207] The amount of phenylalanine used for protein synthesis varies considerably with age, although it has been shown that protein synthesis from phenylalanine in phenylketonuria is similar to that in nonphenylketonuric persons.[208]

The major pathway in metabolism of phenylalanine is formation of tyrosine by hydroxylation (Figure 1). With dysfunction of this reaction there is a great increase in the substances that result from alternate pathways of phenylalanine metabolism such as phenylpyruvic acid, phenylacetic acid and phenyllactic acid. A toxic mechanism has long been suspected as playing a role in the pathogenesis of phenylketonuria. Clinical observations tend to confirm that a toxic effect influences the somatic and central nervous system functions which are responsive to changes in the level of phenylalanine intake.[5,53,55,56,209,210]

The concentration of phenylalanine in the free amino acid pool is increased in the presence of phenylketonuria.[211] Of particular interest is that a high concentration of phenylalanine is also associated with disturbances in cerebral serotonin catecholamine metabolism[212] and may cause hypervolemia[213] and, possibly, hypoglycemia.[214]

The biochemistry and its pathogenic implications are well reviewed by Lutz[207] and Brady.[215] Although many facts have accumulated concerning the interdependence of single metabolites, the problem of whether there is a key reaction which explains the brain damage is still unresolved.

The permanent nature of impairment in untreated patients affected with phenylketonuria has led to a search for damage to structural elements of the brain. The most consistent pathologic feature in untreated phenylketonuria has been moderate reduction in the weight of the brain,[216] which is in keeping with the well-known frequency of microcephaly.[217-218] This finding has been reversed with treatment where microcephaly is not the expected diagnosis.[5,56,59,82,83,109,219]

Much attention has been given to the formation of myelin. The implication is that a metabolic defect causes interference with the proper formation of myelin or myelin proteins. It is known that phenylalanine can interfere with myelin protein under certain experimental conditions;[220,221] however, this concept may be overused because only small focal areas of demyelination have been found consistently in phenylketonuric patients.[217]

It has recently been noted that heterozygotes for the PKU trait, who have never had elevated phenylalanine levels, frequently present with intelligence quotients below normal.[210,222] Thalhammer and colleagues[210] pointed out that tyrosine levels are normal in these persons. They speculated that the genes for PKU may have some direct role in the growth of brain cells, and thereby influence intellectual development by means other than elevation of phenylalanine levels in the blood.

Conclusion

New information concerning the biochemistry of phenylalanine metabolism has accumulated over the past few years, yet the cause of mental retardation in untreated classic phenylketonuria remains obscure. Although mental retardation is not an expected outcome, several studies show that even in carefully treated patients intellectual development may be several intelligence quotient points lower than expected. Whether this represents an effect of low levels of phenylalanine or its metabolites or some genetic influence on intellectual development remains unclear.

An elevated level of serum phenylalanine is indicative of a diagnosis of phenylketonuria; however, loading studies are necessary to confirm the diagnosis. Children with phenylalanine levels consistently above 10 mg per dl should be treated until clinically acceptable levels are reached.

Current information about phenylalanine metabolism indicates that the defect in about 3 percent of all apparent classic phenylketonuria is, in

fact, due to dihydropteridine reductase or biopterin deficiency. Clinically, these varieties can not be distinguished; however, hypotonicity, convulsions and continued deterioration during adequate dietary therapy suggest this diagnosis.

The chance of missing a diagnosis of phenylketonuria is greatest in neonates; therefore, it is desirable to screen newborn infants as late as possible before they are discharged from hospital.[223] For those babies in whom results were negative before 3 days of age, a second determination of the phenylalanine level should be done at the first well-baby visit.

The isoenzymes of phenylalanine hydroxylase tend to complicate the situation further, although isoenzyme activation may explain why some neonates present with classic phenylketonuria at 3 weeks of age, but at a year have a tolerance test showing only mild hyperphenylalaninemia. Isoenzymes may also play a role in the high percentage of affected offspring from persons with hyperphenylalaninemia as well as phenylketonuria.

Because there is increased incidence of pyloric stenosis in those with phenylketonuria, infants with excessive vomiting or proved pyloric stenosis should have a repeat examination of levels of phenylalanine in the blood.

The problem of when to discontinue a low-phenylalanine diet is still unsettled. Some investigators recommend stopping at age 4 to 5 years; others recommend continuing the diet throughout the patient's lifetime. Most physicians now agree that the special diet should not be stopped before the age of 6 years.

Probably of greatest concern is maternal hyperphenylalaninemia. Most children born to mothers with this disorder are mentally retarded. Dietary restriction of phenylalanine and supplementation with tyrosine is the only treatment available, and its effectiveness remains to be confirmed.

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